2. Literature review

2.1. P-gp Efflux System

The ultimate goal of introducing novel dosage form or drug delivery system is to attain longer plasma drug concentration at desired level in blood, target to specific site and reduce the toxic effects of drug. Among different routes of administration, oral route is most preferable due to numerous advantages such as ease of administration, storage and economy (Gupta *et al*., 2009). Due to these exceptional benefits, researchers are incessantly trying to develop novel dosage form for oral route administration to treat various diseases. During the development of new dosage forms, formulation scientists are need to consider various factors such as physicochemical properties of drug, nature of excipients, preparation techniques, methods to carry out *in-vitro* and *in-vivo* studies (Boxenbaum, 1982). In spite of following all these specifications in development of ideal dosage form, in several occasions the goal like retaining the desired plasma level, intracellular concentration and appropriate bio-distribution of drug at targeted site is not met. In this circumstance, the practice of increases in dose strength, dose frequency and prolongation of drug therapy lead to severe adverse and side effects.

The constant efforts of researchers on reasons for failure of drug therapy provided more valuable information regarding interaction of drug with

transporters at membrane level. Interestingly, researchers have found one of the membrane protein, phosphoglycoprotein (P-gp) as a transporter which causes efflux i.e. transport of its substrates from intracellular to extracellular level (Sharom, 1997). Some potent therapeutic drugs such as paclitaxel, doxorubicin and saquinavir (P-gp substrates) are susceptible to P-gp efflux. The P-gp efflux may possibly alter the pharmacokinetic profile of drug which ultimately causes development of multi drug resistance (MDR). Several excellent reviews have described the influence of P-gp efflux and their inhibition by certain P-gp inhibitors in order to improve the bioavailability of P-gp substrates (Akhtar *et al*., 2011).

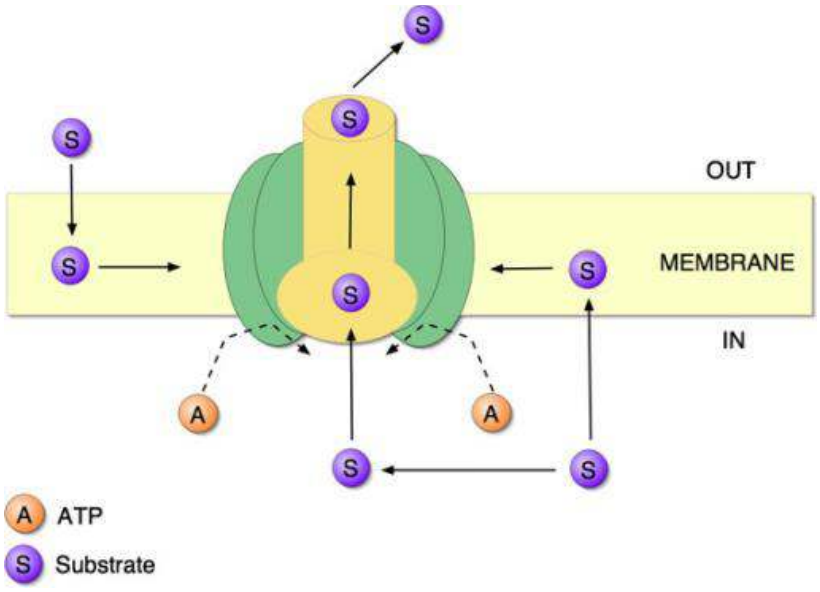


Figure 2.1. Schematic representation of P-gp efflux

2.1.1. Outline of P-glycoprotein

Phosphoglycoprotein (P-gp) is discovered in 1970 as a transporter involved in multi drug resistance of cancer cells (Juliano and Ling, 1976). It is belongs to super family of Adenosine Tri Phosphate (ATP) binding cassette (ABC) transporter which consist of 1280 amino acids, molecular weight of 70 KDa composed by a single chain containing two homologous portions of equal length, each consist of six trans membrane domains and two ATP binding regions separated by a flexible poly peptide portion (Schinkel *et al*., 1993; Ambudkar *et al*., 1999). In human beings P-gp is widely distributed in columnar epithelial cells (enterocytes) at lower gastrointestinal tract (GIT), canalicular surface of hepatocytes, apical surface of proximal tubules, capillary endothelial cells of brain, testis and in pregnant uterus (Ambudkar *et al*., 2006). Generally, P-gp does not shows any specific role in the vital body functions but on the basis of interaction with foreign bodies at membrane level it may be considered that P-gp plays a protective role in the body, for example active transport of cytotoxic compounds and endotoxins from intercellular to extracellular level and exportation of xenobiotics (Thiebaut *et al*., 1987; Fromm, 2004).

2.1.2. Factors affecting P-gp efflux

Usually a number of factors are involved in admission of drug from dosage form for exhibiting of desired therapeutic efficacy. Number of drugs either natural, semi synthetic or synthetic compounds have been

reported as substrates of P-gp (Varma *et al*., 2003). Also should accept that the P-gp does not show effect on the bioavailability of all categories of therapeutically valuable drugs (Chiou *et al*., 2001; Kuppens *et al*., 2005). However, the P-gp efflux is possibly affected by factors such as biopharmaceutical character of drug and variables in GIT physiological system (Di and Kerns, 2003; Mouly and Paine, 2003).

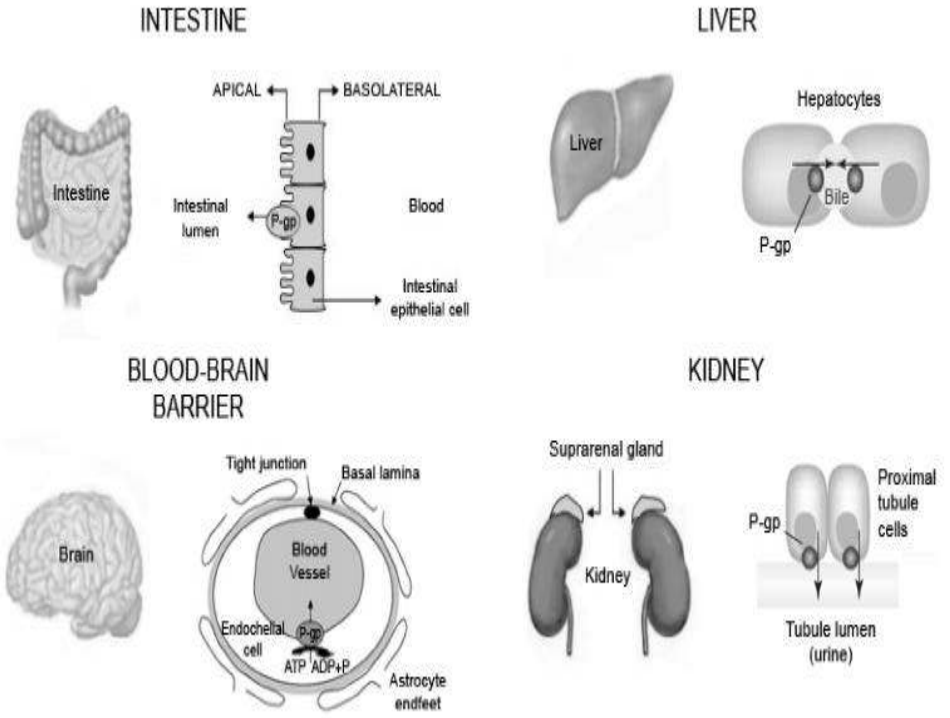


Figure 2.2. P-gp in different organs of the body

Among the above mentioned factors related to P-gp efflux, Biopharmaceutical character of drug such as molecular weight, partition coefficient and presence of number of hydrogen bond acceptor and

donors are of primary of importance. The physicochemical properties of drug are required to meet Lipinski's rule for development of suitable dosage form, as per that rule a drug should have the following properties: 1. Molecular weight (MW) < 500 Da, 2. Number of hydrogen bond acceptors <10 3. Number of hydrogen bond donors <5 4. Partition coefficient log P <5 (Lipinski, 2000). Molecular weight and partition coefficient plays an instrumental role in deciding of solubility and permeability nature of drug. A research group has done work on characterization of P-gp substrates from non P-gp substrates based on the molecular weight, number of hydrogen bond acceptor, donors. Interestingly, they were found that P-gp substrates have not followed certain limits of above mentioned five rules theory (Didziapetris *et al*., 2003). Another research study has reported the contribution of passive permeability on P-gp efflux. In that study, they have illustrated the molecular weight and partition coefficient corresponding passive permeability nature of various P-gp substrates. It was observed that the P-gp substrates having molecular weight < 500, log P in limit of 1- 5 and high permeability were not susceptible to efflux compared to P-gp substrates with molecular weight > 500, higher log P then above mentioned limit and less permeability. Also, the author's suggested less or moderate permeability nature of drug molecule more prone to P-gp efflux (Varma *et al*., 2005).

The other important factor in P-gp efflux is variables in physiological system such as intestinal transit time, pH of intestinal fluid and degree of ionization. P-gp is very specifically distributed in various organs of our body such as intestinal lumen, liver, kidney, brain, testes and in placenta. In density versa more expression of P-gp efflux exhibited at distal region due to abundantly presence of P-gp at distal region compare to proximal region of organ of body (Stephens *et al*., 2002). It means the gradient distribution of P-gp, from proximal to distal region of intestine (duodenum to ileum region). The high level expression of P-gp at the ileum region, intestinal lumen part is the probable reason for significant reduction in the absorption or permeation of drugs. Commonly, orally delivered drugs are absorbed or permeated from different regions of gastro intestinal tract. The drugs which are absorbed from stomach region are not susceptible to P-gp efflux whereas the drugs which are absorbed through different intestinal region are prone to P-gp efflux at their absorption site. In this case, the relation of drug molecule with intestinal transit time and ionization at pH of intestinal fluid should be considered. The highly permeable drugs are easily absorbed from jejunum region within the appropriate transit time ~ 40 minutes because it may rapidly partition to membrane and balance the P-gp efflux transport by the maximum passive influx. However, poorly soluble drugs change their absorption or permeation site towards ileum region, which

has a transit time of ~ 140 minutes due to peristaltic movement of GIT. Thus, the drugs permeated from ileum region are more exposed to efflux system due to higher expression of P-gp at ileum region of intestinal lumen (Varma *et al*., 2005 a). It has been reported that pH of GIT fluids facilitates favorable conditions of drug ionization at particular absorption site could enhance the bioavailability of drug (Neuhoff *et al.*, 2003; Neuhoff *et al.,* 2005). Few researchers have demonstrated the influence of pH and relative changes in ionization of drug on permeability property at high expression site of P-gp, i.e. ileum. In one of the study found that increased solubility of quindine at pH of intestinal ileum may overcome the susceptibility of P-gp efflux due to the higher passive influx of quindine (Varma and Panchagnula, 2005).

2.1.3. Mechanism involved in P-gp efflux

Different types of P-gp efflux models have proposed to explain the mechanism of exportation of drug molecule by P-gp, However, till to date the specific site of interaction of drug molecule with P-gp is not clearly understood. The investigational efforts of researchers have proposed three promising models, such as pore model, flippase model and hydrophobic vacuum cleaner (HVC) model to describe the P-gp efflux mechanism.

Pore model, initially invokes pore arrangement at the transmembrane domain (TMD) initiate the exportation of drug molecule from cytoplasm to extracellular location by P-gp (Borst and Schinkel, 1997). The proposed flippase model, P-gp substrate shows more affinity towards P-gp and its binding at the planner transmembrane domain (TMD) of protein causes its translocation to outer leaflet bilayer from inner leaflet of bilayer membrane from which they are passively diffused into extracellular fluid (Higgins and Gottesman, 1992). Hydrophobic vacuum cleaner (HVC) model has been widely accepted as this model explains about exportation of substrate through P-gp from intracellular to extracellular level has been possible by pore as well as filppase of molecule (Sauna *et al*., 2001). However, for weak cationic and lipophilic basic drugs, another model has been proposed, it is an indirect P-gp efflux which plays a secondary role of transport system, It alters the pH of intracellular region or membrane potential functioning as a proton or chloride pump and reduces the accumulation of substrate molecule (Fardel *et al*., 1996).

2.1.4. Approaches to overcome P-gp efflux

A significant role of P-gp efflux in alteration of drug kinetics and influence on drug therapy was found in various clinical investigations. Therefore, the pharmaceutical scientists are following certain approaches to avoid

influence of P-gp efflux on drug. The P-gp efflux can be overcome by using the following methods (Bansal *et al*., 2009; Bansal *et al*., 2009 a).

1. Development of novel non P-gp substrates
2. Co-administration of P-gp inhibitors with drug
3. Designing formulations that allow the drug to bypass P-gp efflux

Among the above methods, the first method is quite tedious and may take more time and money for developing a new chemical entity consisting of non P-gp substrate character. However, the second and third methods are more feasible and also widely accepted due to implementation of polymer technology and nanotechnology in the development of various novel drug delivery systems.

2.1.5. Overview on various generations of P-gp inhibitors

The compounds which are capable of transporting of P-gp substrates i.e bypass P-gp efflux are known as P-gp inhibitors and other synonyms are chemo sensitizers, P-gp modulators (Litman *et al*., 2001). Prediction and utilization of P-gp inhibitors could make better opportunity to overcome the issues with P-gp efflux in drug delivery. Various P-gp inhibitors were discovered in past two decades based on their specificity, affinity and potency (Krishna and Mayer, 2000). They are categorized as follows:

2.1.5.1. First generation P-gp inhibitors

First generation inhibitors, sum of the compounds owing pharmacological actions and used in clinical practice for their respective indications as very well known compounds such as calcium channel blockers - verapamil, immune suppressants - cyclosporine A and cardiovascular drugs - reserpine and quinidine. Numerous studies have demonstrated the possible use of first generation P-gp inhibitors that overcome the P-gp efflux. One of study demonstrated that verapamil at non cytotoxic dose act as a P-gp inhibitor and enhanced *in vitro* and *in vivo* cytotoxicity of vincristine and vinblastine in P388 leukemia cells (Tsuruo *et al*., 1981). In another study, verapamil at 1 mM concentration dramatically increased the digoxin absorption from duodenum and proximal ileum region of intestinal tract but it is insignificant on absorption of digoxin at colon site. It was explained verapamil is inhibited the digoxin efflux by P-gp only at intestinal region (Sababi *et al*., 2001). The phase 1 clinical trial conducted for the determination of etopside pharmacokinetic and dynamics in presence of cyclosporine as a P-gp modulator. In this study, etopside alone and in combination of high dose of cyclosporine infusion administered to 16 patients and evaluated the various kinetic parameters. The results of that clinical trial illustrated cyclosporine with etopside administration causes marked changes in kinetics of etopside (Lum *et al*., 1992; Erlichman *et al*., 1993). However,

the usage of first generation inhibitors are very limited because P-gp inhibition is achieved at higher dose level which may leads to toxicity.

2.1.5.2. Second generation P-gp inhibitors

Second generation P-gp inhibitors, which resembles like first generation compounds but lack of pharmacological activity and possess higher affinity towards P-gp, These compounds included cyclosporine D, R-verapamil, valspodar (PSC833), quinidine analog MS-209, S-9788, GF-12918, and VX-710 (biricodar) (Robert *et al*., 2003). One clinical study, R-verapamil and paclitaxel alone and combination doses were administered 3 days to six breast cancer patients and observed the pharmacokinetic profile of paclitaxel. In this case they were found the significantly increased paclitaxel concentration in plasma by decrease the disposition rate of drug due to P-gp reversal property of R- verapamil (Berg *et al*., 1995; Tolcher *et al.*, 1996). Another phase I clinical study, patients with solid malignancies received VX-710 (biricodar) doses that ranged from 10 to 120 mg per hour administered as a 24-hour infusion. After a 2-day washout period, patients again received VX-710 with schedule dose followed 8 hours later by paclitaxel doses that ranged from 20 to 80 mg as a 3-hour infusion. Thereafter, in during treatment period examined the pharmacokinetics of alone and combination of both VX-710 and paclitaxel. The results indicated that the combination of paclitaxel and P-

gp modulator (VX-710) could be useful for maintenance of steady state level of paclitaxel in plasma (Rowinsky *et al*., 1998). The studies on effect of PSC 833 on accumulation of epirubicin in Caco 2 cells demonstrated the significant accumulation of epirubicin from apical to baso lateral side in caco 2 cells. Finally, they were concluded combination of PSC 833 either in free form or lipid formulation such as liposome useful for avoiding of drug resistance in cancer therapy (Lo *et al*., 2001). Second generation compounds also have limited use as P-gp inhibitors due to either reversible or irreversible drug-drug interaction which finally results in decreased therapeutic efficacy of drugs (Baer *et al*., 2002).

2.1.5.3. Third generation P-gp inhibitors

The draw backs related to the above respective generations, explains the challenges in development and usage of highly potent P-gp inhibition compounds. In this view, some of the compounds such as elacridar, LY-335979 (zosuquidar), R101933 (laniquidar), OC144093 and XR9576 (tariquidar) developed as a third generation compounds (Pajeva and Wiese, 2009). One of the study, GF120918 (25 mg/kg) was administered by oral before oral dosing of paclitaxel in both wild-type and mdr1ab knockout mice. GF120918 significantly increased the oral bioavailability of paclitaxel in wild-type mice whereas it is insignificant in case of mdr1ab knockout mice. They were concluded, GF120918 at 25 mg/kg dose-level selectively and completely blocks P-glycoprotein without alteration of

elimination of paciltaxel (Hyafil *et al.,* 1993; Bardelmeijer et al., 2000; Guns *et al.*, 2002). In clinical study, forty patients received zosuquidar. trihydrochloride (LY335979) and doxorubicin intravenously separately in cycle 1 and concomitantly in cycle 2 over 48 h and 0.5 h, respectively. Significant changes of doxorubicin and doxorubicinol (metabolite) kinetic parameters such as clearance (CL), peripheral volume of distribution (Vd), apparent clearance (CLm/fm) and apparent volume of distribution (Vm/fm) in presence of high doses (≥ 500 mg) of LY335979 was observed (Dantzig *et al*., 1996; Callies *et al*., 2003). In another clinical study, examined the disposition of docetaxel with and without R101933 (P-gp inhibitor) in nine patients. The results demonstrated combination of docetaxel and R101933 causes decreased fecal excretion of docetaxel due to P-gp inhibition of R101933 (Van *et al*., 2002). In spite of P-gp inhibition property, these compounds are also exhibit unpredictable drug interactions leads to acute or chronic toxicity.

2.1.5.4. Novel P-gp inhibitors

However, the P-gp inhibitors of the above three generations do not meet the ideal properties of P-gp inhibition such as high affinity to P-gp, less quantity, absence of drug interaction, no interference at plasma concentration and toxicity. Interestingly, some pharmaceutical excipients have shown ability to inhibit P-gp efflux. Pharmaceutical excipients such as some polymers and surfactants are considered as a novel generation P-

gp inhibitors. The study was conducted on certain pharmaceutical excipients such as poly (ethylene) glycol (PEG) 300, Cremophor EL and Tween 80 for evaluation of their P-gp inhibition property in Caco-2 and MDR1- transfected Madin Darby Canine Kidney (MDR1-MDCK) cell. In this study PEG-300 at (20%, v/v) causes complete inhibition of P-gp activity in both Caco-2 and MDR1-MDCK cell whereas Cremophor EL (0.1%, w/v) and Tween 80 (0.05%, w/v) partially inhibit P-gp activity in Caco-2 cells and almost inactive as P-gp inhibitors in MDR1-MDCK cell due to the differences in interaction of excipient surface and cell membrane. However, the results are confirmed the P-gp inhibition property of certain pharmaceutical excipients (Hugger *et al*., 2002; Shono *et al*., 2004). Some other studies on these excipients also demonstrated P-gp efflux inhibition property.

2.1.6. Various mechanisms of P-gp efflux inhibitors

In general, P-gp can be inhibited by mechanisms such as (i) blocking of drug binding site either competitively, non- competitively or allosterically (ii) interfering of ATP hydrolysis and (iii) changing integrity of cell membrane lipids (See *et al*., 1974). The most accepted mechanism is inhibition of P-gp by blocking the substrate binding site, The response of P-gp on compounds either substrates or inhibitors for importation or exportation occurs only after binding of substrates through the

appropriate binding sites. Moreover, the presence of multiple binding sites and affinity of P-gp substrates create a problem in order to understand the binding site blocking mechanism at molecular level. However, one of the study proposed an explanation by flip – flop model for better understanding of the P-gp inhibition mechanism. In this model P-gp merely act as a flippase to export the substrate from intracellular to extracellular region of bilayer membrane as well as import the inhibitor by flop i.e back into intracellular site for further transport. The flop step occurred due to hydrophobic character, difference in rate of efflux between substrates and inhibitor compounds (Eytan *et al*., 1996). From absorption point of view the high affinity and moderate or less permeable compounds such as verapamil, tanilol respectively support this model (Doppenschmitt *et al*, 1999).

The inhibition of ATP hydrolysis is one of the most reliable mechanisms of P-gp efflux. These types of compounds require very less quantity and might be possible by acting at local gut wall region only. Till date, there is difference in opinion regarding the mechanism of inhibition of ATP hydrolysis. It is still not clear whether the inhibition is due to binding of the ATP molecule or direct interference of ATP hydrolysis at ATP binding site. At the same time it is also not clear whether the compounds interfere with the nucleotide binding site in order to inhibit P-gp activity. Few researchers proposed certain mechanism of inhibition of ATP hydrolysis

related to multiple binding sites and orientations of substrate or inhibitor coupling to the P-gp at molecular level. However, the unavailability of exact model illustrating inhibition mechanism of ATP hydrolysis restricts the predicting of the ability of some clinically potential P-gp inhibitors.

The third generation P-gp inhibitors exhibited different types of mechanism of P-gp inhibition such as change in the integrity of membrane lipids, change of structure moiety and change in fluidity of cell membrane. Excipients such as surfactants like Tween 80, Cremophor EL change the fluidity of cell membrane facilitates the importation of P-gp substrates.

2.1.7. Toxicity issues of inhibition of P-gp efflux

Usually, administrations of higher doses, dosage frequency and combined drug therapy have been responsible for drug interactions and toxicity. The maintenance of drug concentration balance between efficacy and toxicity is very sensitive because, the particular quantity of drug available in plasma has been responsible for the showing of either effect or toxicity. The improvement of the therapeutic efficacy of P-gp substrate drugs achieved by incorporation of P-gp inhibitors in drug delivery systems. This could be occurs either by increasing the rate of absorption, distribution or delaying of metabolism and excretion (ADME). The alteration of ADME may cause adverse effects and toxicity of drugs. Few

researchers have been reported, the administration of P-gp inhibitors PSC 833 and XR9576 along with P-gp substrate doxorubicin as an anti cancer drug resulting in an increased chances of mucositis. Few studies explain about toxicity of doxorubicin due to alteration of metabolism. Doxorubicin has been co administered with some P-gp inhibitors as a result leads to delaying of metabolism and accumulation of metabolic product such as doxorubicinol causes the neutropenia (Advani *et al.,* 2005). Another study also confirmed toxicity of Doxorubicin in terms of pharmacokinetic and dynamic relationship between doxorubicinol and neutropenia. Similarly, vincrisitine; anti cancer drug, the P-gp inhibition at blood brain barrier allows more drug penetration in to CNS region initiate the indirect toxicity such as irreversible cerebellar damage. In consideration of drug disposition, metabolism process, metabolic products were addressed as a mediator of toxicity.

It may conclude that the main reasons for reported toxicity of P-gp substrates due to the administration with higher amount of P-gp inhibitors or drug interactions. Thus, exploring of novel P-gp inhibitors such as inert pharmaceutical exicipients and application of nanotechnology in delivery of P-gp substrates may be beneficial. It has been reported the novel drug delivery systems such as liposomes, nanoparticles, nanosponges, nanocolloids and nanocarriers more beneficial in terms of enhancement of therapeutic efficacy, targeting at

site, controlled release and reducing of toxicity as compare to the existed conventional dosage forms.

However, we should aware that the formulations containing P-gp inhibitors may cause partial or reversible disturbance in the physiological system of the body. Therefore, it is important to carry out an in depth mechanistic toxicological evaluation for such developed novel drug delivery systems.

2.2. Berberine Chloride

Berberine is a plant alkaloid with a long traditional history and is used both in Ayurveda and Chinese medicine. This alkaloid is present in many plants, including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Tinospora cordifolia, Arcangelisia flava*, *Cortex rhellodendri, Rhizoma coptidis, Coptis japonica, Thalictrum* minus, *Berberis wilsonae* and *Berberis aristata* (tree turmeric). This phytochemical constituent can be found in the root, rhizome and stem bark of the plants. As a drug, it is traditionally used for its antimicrobial and antiprotozoal properties in Ayurveda, Chinese and Middle-East folk medicine. Specifically, Ayurveda describes berberine extracts and decoctions to have significant antimicrobial activity against a variety of organisms including bacteria, virus, fungi, protozoa, helminthes and Chlamydia (Arayne *et al*., 2007). Interestingly, current clinical research on berberine

have revealed various other pharmacological properties and medicinal uses which are beneficial in treatment of chronic ailments or diseases including diabetes, cancer, depression, hypertension and hypercholesterolemia (Imanshahidi and Hosseinzadeh, 2008).

Figure 2.3. Berberine species

Figure 2.4. Berberine species bark

Figure 2.5. Berberine chloride (pure form)

2.2.1. Traditional uses

2.2.1.1. Anti microbial activity

Berberine usually available as chloride and sulfate salts has a high bacteriostatic activity against *Staphylococcus epidermis, Neisseria meningitids* and *Escherichia coli.* One study has shown that berberine is specifically effective against cholera, giardia, shigella, and salmonella (Chevalier, 2001). In another study, which supports the anti microbial activity of berberine, has evaluated the microbial effect of the drug against 17 gram positive and gram negative microorganisms on the basis of inhibitory concentration (IC 50), minimum inhibitory concentration (MIC), minimum microbicidal concentration (MMC) and minimum microbistatic concentration (MMS). The IC 50 value obtained for *S. Aureus* was 14.6 mg/ml while for B. Subtilis it was 43 mg/ml. The high value in the later case could be attributed to the development of resistance by the spores. The above study shows that berberine has marginal action on

both gram positive and gram negative organisms (Cernakova and Kostalova, 2002). Materia Medica, a compilation of Chinese herbal medicines, indicates that berberine sulfate demonstrates significant antimicrobial activity against a wide range of micro-organisms, including *Staphylococcus* (Staph)*, Streptococcus* (Strep)*, Candida and Salmonella,* as well as *klebsiella, clostridium, pseudomonas proteus, shigella, vibrio, cryptococcus* and *entamoeba* species (Bensky *et al*., 1993). The National Institutes of Health has also reported that extracts of berberine have demonstrated significant antimicrobial activity against bacteria, fungi, viruses and chlamydia which confirms the antimicrobial activity of the drug (Sack and Froehlich, 2000).

2.2.1.2. Anti protozoal activity

Berberine extracts and salts have demonstrated growth inhibition of *Giardia lamblia, Trichomonas vaginalis* and *Leishmania donovani* (Kaneda *et al.,* 1991; Ghosh *et al*., 1985). The crude extracts of berberine have shown to be more effective than its salts. In tropical climates, *Giardia lamblia* infestation (giardiasis) is a common occurrence, particularly in pediatric population (Nair, 1973). In a clinical trial, berberine administration improved gastrointestinal symptoms and resulted in a marked reduction in Giardia-positive stools and was effective at half the dose of the popular giardiasis medication, metronidazole. A study has revealed the drug's ability to markedly inhibit parasitic load and rapidly

improve hematologic parameters in infected animals. *In vitro* results indicate that the drug has the ability to suppress organism maturation through inhibition of its multiplication, respiration and macromolecular biosynthesis of amastigote forms of the parasite, and interference with nuclear DNA of the promastigote form. A randomized clinical trial on 215 patients has shown that pyrimethamine effect on chloroquine resistant malaria was increased more by berberine (74%) than by tetracycline (67%) or cotrimoxazole (48%) which indicates its antimalarial activity, as well (Sheng *et al.,* 1987).

2.2.2. Medicinal applications

2.2.2.1. Anti diarrheal activity

Diarrhea caused by *Vibrio cholera* and *Escherichia coli* has been the focus of numerous studies on berberine, and results indicate several mechanisms that may explain its ability to inhibit bacterial diarrhea. Berberine has been found to reduce the intestinal secretion of water and electrolytes induced by cholera toxin (Swabb *et al*., 1981). Other studies have shown that berberine directly inhibits some *V. cholera* and *E. coli* enterotoxins significantly, reduces smooth muscle contraction, intestinal motility and delays intestinal transit time in humans. *In vitro* study indicates that berberine sulfate inhibits bacterial adherence to mucosal or epithelial surfaces which is the first step in the infective process. This may be a result of berberine inhibitory effect on fimbrial structure

formation on the surface of the bacteria (Sun *et al*., 1988). Another study on mice has shown that berberine has some activity against *E. histolytica*. This property makes it useful against bilious disorders.

2.2.2.2. Anti cancer activity

In order to investigate the anti-carcinogenic activity, the killing effect of berberine on nasopharyngeal carcinoma cells (NPC/HK1) was investigated. In this experiment, cytotoxic effect of berberine in cell lines was assessed by using trypan blue exclusion assay. Surprisingly, berberine, at 5-200 μM concentration was found to induce cell death in a dose dependent manner. Treatment of cells with 200 μM concentration of berberine for 5 hours yielded a lethal dose of 50% (LD 50). The extent of DNA damage and repair after berberine treatment (0 -100 μM) was also evaluated, using comet assay. Administration of berberine up to 200 μM caused un-repairable damage of the cells, as was indicated by the increase in tail DNA content. However, the repair of DNA damage on this cell line in presence of H_2O_2 occurred within 1.5 h, indicating that berberine has contributed in the process of DNA repair inhibition which finally resulted in the cell death (Szeto *et al.,* 2002).

An *in vitro* cell viability study demonstrated that berberine improves As_2O_3-mediated inhibition of glioma cell growth after 24 hours incubation. Here, the formation of a confluent layer of untreated control cells was observed which was inhibited upon incubation with 5 μM

concentration of As_2O_3. The latter effect was even more pronounced in the presence of 10 μM concentration of the drug. The As_2O_3-mediated reduction in motility and invasion of glioma cells was enhanced upon co-treatment with berberine. Furthermore, it has been reported that PKC isoforms influence the morphology of the actin cytoskeleton, as well as the activation of metalloproteases, MT1-MMP and MMP-2, which play a key role in cancer cell migration. In this case also treatment of glioma cells with As_2O_3 and berberine, significantly, decreased the activation of PKC α and ε due to actin cytoskeleton rearrangements and blocking of the PKC-mediated signaling pathway. The result of this work is interesting as it indicates the potential use of berberine as a novel chemotherapeutic agent in the treatment of malignancy (Lin *et al.,* 2008).

2.2.2.3. Anti diabetic activity

Berberine has shown marked impact on carbohydrate and lipid metabolism. Recent preclinical and clinical studies suggest that it has a strong impact on glucose homeostasis. In fact, berberine increases insulin receptor mRNA expression through protein kinase C-dependent as promoter in cultured human liver cells and skeletal muscle (Kong *et al*., 2009).

Berberine has shown to provide protection against β cell damage and protection of pancreas from oxidative stress in diabetic rats. In an animal study, diabetic and hyperlipidemic condition was induced in rats by intra-

peritoneal injection of 35 mg/kg streptozotocin and administration of high-carbohydrate/high-fat diet. The overall experiment was conducted by using seven groups of rats consisting of diabetic untreated and treated rats with 75/150/300 mg/kg berberine, rosiglitazone 4 mg/kg and fenofibrate 100 mg/kg, and a control group. After 16 weeks of treatment, estimation of serum insulin level, insulin expression in pancreas, malonaldehyde content and superoxide dismutase activity in pancreas was carried out. It was observed that the diabetic rats showed alteration in pancreas to body weight ratio, insulin level, insulin sensitivity index, malonaldehyde content and superoxide dismutase activity. On the other hand, rats treated with 150 and 300 mg/kg berberine showed near control levels in the evaluating parameters. Moreover, in diabetic rat's mitochondrial vacuolization, swelling and dilatation of β cells endoplasmic reticulum in pancreas was observed. The pancreatic islets were found to be atrophied and the number of secretory granules was decreased in diabetic rats but less pathological changes was observed in rats treated with 150 and 300 mg/kg berberine. These findings strongly suggest that berberine has protective effect for diabetes by the process of increasing insulin expression, β cell regeneration, antioxidant enzyme activity and decreasing lipid peroxidation (Zhou *et al*., 2009).

Yin and colleagues have demonstrated scientific evidence for the use of berberine in human beings to treat type 2 diabetes mellitus (Yin *et al.,*

2008). This clinical study was conducted on two age groups with type-2 diabetes mellitus. In study A, 36 adult subjects were selected with newly diagnosed type 2 diabetes mellitus and were randomly assigned to treatment with berberine or metformin (0.5g 3 times a day) for a period of 3 months. Periodically, the hemoglobin A1c, fasting blood glucose, postprandial blood glucose and plasma triglycerides were estimated. Significant decrease in hemoglobin A1c (from 9.5% ± 0.5% to7.5% ± 0.4%, $P < .01$), fasting blood glucose (from 10.6 ± 0.9mmol/L to 6.9 ± 0.5mmol/L, $P <.01$), postprandial blood glucose (from 19.8 ± 1.7 to 11.1 ± 0.9 mmol/L, $P<.01$) and plasma triglycerides (from 1.13 ± 0.13 to 0.89 ± 0.03mmol/L, $P < .05$) were observed in the berberine group. The drug showed similar hypoglycemic effect to that of metformin. In study B, 48 adult subjects with poorly controlled type 2 diabetes mellitus were supplemented with berberine for a period of three months and were clinically evaluated, similar to that in study A. Berberine was found to significantly decrease fasting blood glucose and postprandial blood glucose from the first week till the end of the trial. Hemoglobin A1c decreased from 8.1% ± 0.2% to 7.3% ± 0.3% ($P < .001$) while fasting plasma insulin and homeostasis model assessment of insulin resistance index were reduced by 28.1% and 44.7% ($P < .001$), respectively. During the study, 20 (34.5%) patients experienced transient gastro intestinal related adverse effects. Physiological functions of liver or kidney damages

were not observed in any patient. Thus, this study indicates that berberine is a potent oral hypoglycemic agent with beneficial effects on lipid metabolism as well.

A recent study has investigated the molecular mechanism of berberine against insulin resistance wherein the drug was found to increase insulin sensitivity through activation of insulin receptor (InsR). Berberine showed a dose-and time-dependent increase of InsR expression, InsR messenger RNA (mRNA) and protein expression in cultured human liver cells and L6 rat skeletal muscle cells. It was also observed that berberine-enhanced InsR expression increases cellular glucose consumption only in the presence of insulin and that berberine promotes InsR gene expression through a protein kinase C (PKC) – dependent activation of its promoter. Inhibition of PKC abolished berberine caused InsR promoter activation and InsR mRNA transcription. In animal models, treatment of type 2 diabetes mellitus affected rats with berberine showed lowered fasting blood glucose and fasting serum insulin, increased insulin sensitivity and elevated InsR mRNA and PKC activity in the liver. In addition, berberine lowered blood glucose in KK-Ay type 2 but not in NOD/Lt type 1 diabetes mellitus affected mice that were insulin deficient. The results suggest that berberine is a unique phytochemical constituent, active against insulin resistance in type 2 diabetes mellitus and metabolic syndrome.

2.2.2.4. Anti depressant activity

Recently, some neuropsychiatric research studies have investigated the effect of berberine on the central nervous system (CNS). Surprisingly, these studies demonstrated that berberine also possess an antidepressant activity (Kulkarni and Dhir, 2007). It was found that the drug affected the signaling pathway of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) which manifested the antidepressant activity of the drug. The antidepressant activity was confirmed by conducting forced-swim test (FST) and tail-suspension test (TST) (Kulkarni and Dhir, 2008). Total immobility period was recorded during a six-minute test. Berberine (5-20 mg/kg, i.p.) produced a reduction in immobility period in both the tests. When berberine (5 mg/kg, i.p.) was co-administered with other atypical antidepressant drugs like mianserine (32 mg/kg, i.p.) or trazodone (2 mg/kg, i.p.), it was found to improve the anti-immobility effect of sub effective doses of the two antidepressants in forced-swim test but did not modify their effects. Berberine (5 mg/kg, i.p.) increased the levels of norepinephrine, serotonin or dopamine in the mouse whole brain which was detected by using neurochemical analysis method.

In yet another study, the effect of berberine in FST and TST on mouse was investigated (Peng *et al*., 2007). In this study, berberine was administered in combination with atypical anti depressants bearing different

mechanism of actions which includes desipramine (noradrenaline (NA) reuptake inhibitor), serotonin (5-HT inhibitor), maprotiline (selective NA reuptake inhibitor), fluoxetine (selective 5-HT reuptake inhibitor) and moclobemide (monoamine oxidase (MAO) A inhibitor). The levels of these neurochemicals in mice striatum, hippocampus and frontal cortex were measured. The results show that berberine (10 and 20 mg/kg, p.o.), significantly, reduced the immobility time during the FST and TST. Furthermore, berberine (20 mg/kg) increased NA and 5-HT levels in the hippocampus and frontal cortex. The research results support the view that berberine exerts antidepressant activity. The antidepressant mechanism of berberine may be related to the increase in NA and 5-HT levels in the hippocampus and frontal cortex.

2.2.2.5. Anti hyperlipidemic activity

The metabolic effects of berberine have been widely investigated during the past years. In lipid metabolism, it has been seen that berberine was capable of lowering lipid concentration by increasing the transcriptional activity of LDLR promoter by a c-Jun N terminal kinase (JNK) pathway and stabilization of hepatic LDL-C receptor (LDLR) by an extracellular signal –regulated kinase (ERK)- dependent pathway (Abidi *et al*., 2005; Lee *et al.,* 2007). Moreover, the influence on 5' adenosine monophosphate kinase (AMPK) and blocking of the mitogen-activated protein kinase (MAPK)/ERK pathway causes inhibition of lipid synthesis.

The anti hyperlipidemic action of berberine has also been confirmed in human beings (Kong *et al*., 2004). Ninty one hypercholesterlemic people (52 males and 39 females) having IIa and IIb types of hyperlipidemia condition were enrolled in a clinical trial. Patients were divided into two groups, one group received 0.5 g of berberine orally twice per day for a period of 3 months while other group was maintained as a control group. At the end of treatment blood samples were collected and fasting serum concentration of cholesterol, triglycerides HDL – c and LDL – c, as well as liver and kidney functions were examined. 63 hypercholesterolemic people showed significantly lowered cholesterol level by 18%, triglycerides by 28% and LDL – c by 20% but no significant change was observed in HDL – c levels. Moreover, berberine had no impact on kidney functions but improved liver function by reducing levels of alanine amino tranferase, aspertate aminotransaminase and gamma glutamyl transpepetidase enzymes. In control group patients showed no changes in any of the parameters examined. The results of the above clinical trial were re-evaluated in subjects who were neither on other drugs or herbs or nor on special diets before or during berberine therapy. In this study, 500 mg of berberine was administered twice a day to 32 hyperlipidemic patients for a period of three months. The anti hyperlipidemic effect of the drug of this group was compared with eleven patients on placebo

treatment. Berberine was found to significantly reduce the total cholesterol by 29%, triglycerides by 35% and LDL-C by 25 %.

In another clinical study berberine and a combination of berberine with policosanol, red yeast extract, folic acid and astaxanthin were orally administered daily to 40 subjects with moderate dyslipidemias divided in two parallel groups each of 20 subjects. After a period of 4 weeks the total cholesterol, LDL, HDL, Non HDL, ApoB, ApoA, Lp(a) and triglycerides were estimated. Both berberine and the combination were found to significantly reduce TC (respectively by 16% and 20%), LDL (by 20% and 25%), ApoB (by 15% and 29%) and TG (by 22% and 26%), and increase HDL (by 6.6% and 5.1%). It may be concluded that food supplements containing natural products such as those studied could be a useful support to diet and life style changes to rectify dyslipidemias and to reduce cardiovascular risk in subjects with moderate mixed dyslipidemias (Cicero *et al.,* 2007).

2.2.2.6. Anti hypertensive activity

Vasorelaxant effect of berberine has been observed in different animal models. Berberine acts on both endothelium and underlying vascular smooth muscle to induce vasorelaxation via multiple cellular mechanisms. Although the mechanism of action of the drug on the vascular system is not clear, it has been proposed that at lower concentrations berberine mediated aortic relaxation appears to be

dependent on its effect on endothelium while at higher concentrations the effects induced by the drug is independent of the presence of intact endothelium. Other mechanisms involved have also been suggested which include angiotensin–converting enzyme (ACE) inhibitor effect, direct release of NO/ cGMP from rat aortic rings, increased sensitivity to the acetylcholine action and activation of k^+ channels (Kang *et al*., 2002; Chiou *et al*., 1991).

A study has described the α_1-adrenoreceptor blocking activity of berberine (Cheng *et al*., 1987). In this study, berberine and prazosin (a known α_1-adrenoreceptor blocker) were used wherein a parallel right shift in the phenylephrine cumulative dose response curves were observed by both the drugs without any change in maximal response. In isolated rat anococcygeus muscle and rabbit aortic strip, the pA2 was measured. pA2 is an empirical measure of the activity (in concentration terms) of an antagonist that is not dependent on how the antagonist acts. The pA2 is determined by measuring the value of the concentration ratio (r) at several antagonist concentrations, allowing an estimate of the antagonist concentration at which (r) would be 2. The pA2 values of berberine were 6.62 and 6.54, respectively, while for prazosin it was 8.46 and 8.31, respectively. These results indicate that berberine has a competitive α_1-adrenoreceptor blocking action similar to that of prazosin.

A clinical study was carried out by 24 – 48 hour ambulatory monitoring of 100 chronic heart failure and ventricular tachyarrhythmia patients (Lau *et al*., 2001). Berberine was found to decrease the frequency and complexity of ventricular premature complexes and increase the left ventricular ejection fraction in chronic heart failure patients. In patients with ventricular tachyarrhythmia, berberine produced 50% or greater reduction in ventricular premature contraction in 62% and 90% or more reduction in 38% of patients.

2.2.2.7. Anti inflammatory activity

Inflammation is caused by prostaglandins (PGs) wherein the cyclooxygenase-2 (COX-2) plays a key role in its synthesis. In an in vivo and in vitro study carried out on wistar rats, berberine showed positive anti inflammatory effect (Kuo *et al*., 2004). In this study, a 12 hour berberine treatment in concentration of 1, 10, and 100 mM in oral cancer cell line OC2 and KB cells showed reduced prostaglandin E2 (PGE2) production dose-dependently with or without 12-O-tetradecanoylphorbol-13-acetate (TPA) (10 nM) induction. This berberine induced effect occurred rapidly after 3 hours as a result of reduced COX-2 protein, but not enzyme activity. These in vitro anti-inflammatory effects were in agreement to the in vivo results where berberine pretreatment of Wister rats inhibited the production of exudates and PGE2 in carrageenan induced air pouch. Finally, the authors

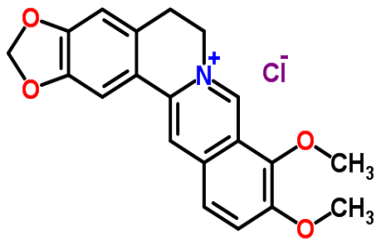
concluded that berberine exhibit the anti inflammatory effect through reduced COX-2 protein but not by the inhibition of enzyme activity.

2.2.3. Current status of therapeutic uses of berberine

Berberine is a traditional medicine, prescribed in Ayurvedic system which has described its use as an anti microbial, anti diarroheal and for treatment of various infections to be administered as kvatha (an Ayurvedic term for decoction) containing 5- 10 ml of drug. Presently, berberine is being used as a dietary supplement in form of 100 – 200 mg capsule dosage form per day in United States but it is not officially approved by US FDA. This drug is also abundantly used in Chinese medicine in dosage forms like tablets and capsule form with 0.2 -1.0 g doses per day for the treatment of various diseases, especially for type 2 diabetes mellitus (Vuddanda *et al*., 2010).

Physico chemical properties of Berberine Chloride (Battu *et al*, 2010; Vuddanda *et al,* 2010)

Appearance	**:**	Crystalline solid
Molecular formula	**:**	$C_{20}H_{18}ClNO_4$
Molecular weight	**:**	371.81
Solubility	**:**	Soluble in water and ethanol
Log p	**:**	-1.28
Chemical name	**:**	5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6 a]quinolizinium chloride
Chemical structure	**:**	



Biopharmaceutical properties of Berberine Chloride

C_{max}	**:**	0.497 µg/ml
T_{max}	**:**	2-3 hr
$T_{1/2}$	**:**	3-6 hr
Bioavailability	**:**	Poor (5 %) due to P-gp efflux inhibition at absorption site
Indications	**:**	Antibacterial, anti protozoal, anti diabetic, anticancer, antidepressant; also used in treatment of cardiovascular disease
Dose	**:**	100 - 200 mg (t.i.d)
Mode of action	**:**	Monoamine oxidase inhibitor and COX-2 inhibitor etc.